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## RAPID FILTRATION OF AGAR AND GELATIN.*

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In the ordinary routine work of a bacteriological laboratory the preparation and filtration of agar and gelatin is probably the most troublesome as well as the most tedious of any technic.

Since Koch revolutionized bacteriological methods, in 1880, by the introduction of the agar-agar culture media, which allowed the satisfactory isolation of organisms, as well as the preparation of stable solid media, workers have been endeavoring to find a more satisfactory and less tedious method of filtration. In many laboratories filtration is carried out by steam or hot water funnels and absorbent cotton, in others the funnel with the absorbent cotton or canton flannel along with the filtering flask is placed in flowing steam. Many do not even go to this trouble and are satisfied with filtering the hot medium through cotton at the room temperature. Drigalski[1] has described "Ein Schnell-filter für Agarlösungen," which is a device for the preparation of agar with the subsequent filtration of the medium in the same apparatus. It gives a large filtering surface and adds thereby to the rapidity of the process.

A method, which has apparently been overlooked, and which is not used in America, as far as the writer knows, was described by

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[1] *Centralbl. f. Bakt., I. Abt.*, Orig., 1906, 41, p. 2.

Bisserie[1] in 1907. The idea involved in his description has been used and modified in our laboratory, and has given us the utmost satisfaction.

After experimenting with many different processes, the simplicity, rapidity, and great efficiency of our modification over other methods has prompted us to bring forward our article. Our procedure in the preparation of 1,000 c.c. of agar is as follows:

A. DOUBLE STRENGTH BROTH.

1. Weigh out—

| | |
|---|---|
| Peptone (Witte) | 10 gm. |
| Sodium chloride | 5 " |
| Meat extract (Liebig's) | 3 " |
| Add—Distilled water | 500 c.c. |

2. Mix together and thoroughly boil for half an hour in a double boiler. "As it is necessary to make the contents of the inner compartment boil, the temperature of the water in the outer compartment must be raised. This is done by using 25 per cent solution of common salt, which raises the boiling point 4.5° C." (Stitt, 1910).

3. Titrate and correct reaction. For neutral agar make this double strength broth neutral. For 0.6+ agar, the broth must be 1.2+, or double the acidity desired in the finished medium. The agar solution, as prepared below, is slightly alkaline or neutral.

Boil again after titration to permit the complete precipitation of the phosphates. Retitrate to control result and to make certain of the stability of the acidity.

4. Filter through ordinary white filter paper. Make up to 500 c.c.

B. AGAR SOLUTION.

| | |
|---|---|
| Agar-agar (for 1.5–2 per cent in finished product) | 15–20 gm. |
| Distilled water | 500 c.c. |

The agar should be finely divided and partly dissolved in the double boiler. (We have found the stick agar cleaner and better

[1] *Ann. de l'Inst. Pasteur*, 1907, 21, p. 235.

than either the thread or the powdered agar.) Place the agar mass in the steam sterilizer and heat to 120° C. for a few minutes. This will give a perfect solution of the agar in the shortest possible time, being decidedly more rapid than when the agar is added directly to the ordinary broth. Moreover, the complete solution is an absolute necessity to insure the success of any method of filtration. Mix together A and B while both are still hot; boil together for a few minutes. Allow to cool to 55°–60° C. Add the whites of two

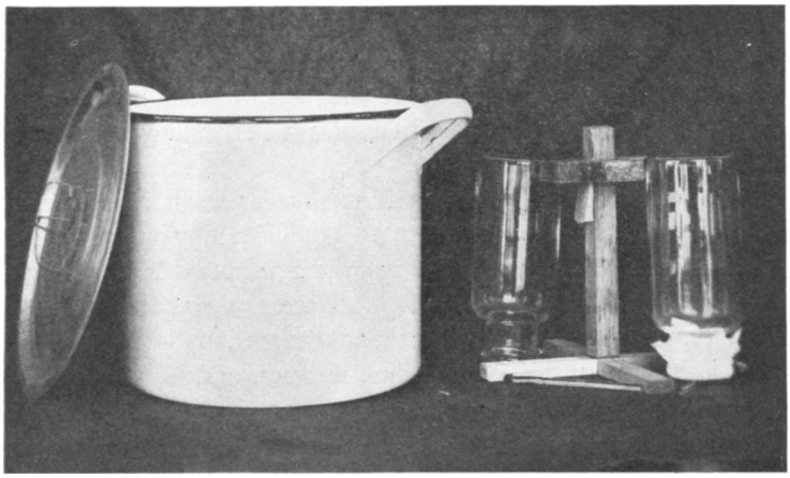

FIG. 1.

eggs well beaten in water to the agar preparation, mix throughly, and pour the mass into the filtering apparatus.

## FILTERING APPARATUS.

The model as used by us is as follows (see Fig. 1):

1. An enameled pot 8.5 inches high by 8 inches in diameter, with a cover.

2. Four ordinary bottles 7 inches high by $2\frac{1}{2}$ inches wide, with a neck $1\frac{1}{2}$–2 inches in diameter (not wider).

3. Canton flannel cut in convenient sizes to cover mouths of bottles.

4. A wooden appliance to steady the bottles when in position.

5. Small glass rods.

The canton flannel is tied firmly over the necks of the glass bottles. The wooden apparatus is placed in the enameled pot containing the prepared nutrient agar. The bottles are put in position with the covered necks downward resting on the glass rods. The cover is put on the pot and the whole placed in the steam sterilizer, heated to 120° C. for a few minutes, to permit a firm coagulation of the egg, and then allowed to cool slowly.

The principle of this filtering method depends on the expansion of the air in the inverted bottles which, bubbling out during the heating, leaves a vacuum on subsequent cooling, and thus exerts a strong suction on the medium, which is slowly drawn into the bottles. The glass rods prevent the bottles from being drawn tightly to the bottom of the container, which would of course stop entirely all further filtration.

The agar prepared and filtered as above is perfectly clear, much whiter than ordinary agar and there is no clouding on subsequent sterilization. By preparing the agar medium in two distinct stages, so that the agar solution and broth are not repeatedly heated together, we find that the darkening of the medium is avoided.

Gelatin is prepared in the usual manner, and it is found that filtration is accomplished equally well by heating it to only 100° C. in the steam sterilizer sufficiently long for the thorough coagulation of the egg.

Glycerin jelly, for use in the mounting of pathological specimens, which offers exceptional difficulty in preparation, is also readily filtered by this method.

We have purposely excluded all metal from this apparatus, having found that small amounts of iron are sometimes absorbed by the hot media, giving color reactions with certain organisms which is undesirable.

The size of the filtering surface we have found to be of some importance. If the vessel has too wide a neck the filtering cover is drawn so far into the interior as to seriously interfere with the complete filtration. If the area is too small the egg may clog the pores of the flannel.

In using wider mouthed vessels, we have employed a perforated metalic disk over which the filtering cloth is tightly drawn.

Although this is quite satisfactory, as far as the filtration goes, we have, for the above reason, preferred not using metals of any kind in our apparatus.

The advantages of the above method are obvious. The final product, whether agar or gelatin, remains much lighter in color, and is devoid of the fine cloud or haziness so commonly obtained by the ordinary methods of filtration. Moreover, the method may be carried on with greater rapidity and with less personal attention. When the filtration is complete there is remarkably little residue. The simplicity of the method makes it adaptable to every laboratory, without the purchase of a special apparatus.